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Enhancement of the Pneumotoxic Effect of Cadmium Acetate by Ionizing Radiation in the Rat

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Experiments involving 120 male Wistar rats were performed to study the effect of treatment with cadmium acetate and external irradiation. A single 0.5 mg/kg body weight dose of cadmium acetate was administered intratracheally. Shortly thereafter, the animals received a single whole-body exposure to 4 Gy γ rays (cesium source). Findings indicated the chemical elevated enzyme activities of lactate dehydrogenase (LDH), alkaline phosphatase (AlP), and acid phosphatase (AP), as well as protein content and percentage of neutrophils in bronchoalveolar lavage fluid (BALF); the percentage of alveolar macrophages was sharply reduced. Radiation alone produced no substantial changes in the parameters investigated. Treatment with both agents combined was found to result in a synergistic rise of LDH, AlP, and AP activities and protein content in BALF. It was concluded that the BALF biochemical markers used are reliable indicators for identifying the type of combined effect produced in the lungs by chemical agents and ionizing radiation.

Introduction

Environmental pollution by toxic substances and the threat of nuclear power plant accidents of the type in Chernobyl are increasing the likelihood of human injuries from combined exposure to chemical agents and ionizing radiation. Our knowledge of mechanisms and characteristics of such injuries is fragmentary. Most reports in the literature have been of *in vitro* studies, and the biological effects examined have largely pertained to the topics of carcinogenesis, mutagenesis, teratogenesis, and cell-killing rates (*1–3*). Other studies have modeled combined injury to tissues as observed in management of malignant disease with cytostatics and high-level doses of ionizing radiation (*4–7*).

Cadmium compounds in general, and cadmium acetate in particular, are highly toxic environmental pollutants. In some areas of Eastern Europe, their levels in the environment are rather high. Acute inhalation exposure to cadmium compounds has been experimentally shown to elicit a lung response proceeding in two phases, with early acute inflammation followed by development of pulmonary fibrosis or emphysema in the regeneration period (*8–13*).

In the work described here, selected biological effects were studied in the lungs of rats treated with a combination of cadmium acetate and ionizing radiation. It was the objective of the experiment to assess lung response by measuring sensitive markers in bronchoalveolar lavage fluid (BALF) and, applying the method of Rothman (*14*), to identify the type of the resulting effects.

Materials and Methods

Experimental Animals. A total of 120 male Wistar rats, 4 months old and weighing on average 200 ± 20 g at the beginning of experiments were used. They were distributed into four treatment groups: group 1, controls; group 2, 4 Gy radiation; group 3, cadmium acetate; and group 4, radiation and cadmium acetate.

Cadmium Acetate. A single intratracheal dose of 0.2 mL cadmium acetate in physiologic saline was administered at 0.5 mg/kg body weight. Control animals were treated in the same manner with 0.2 mL physiologic saline.

Irradiation. Shortly after cadmium acetate treatment, single whole-body exposure to 4 Gy γ rays from a cesium unit was given at a dose rate of 0.91 Gy/min.

Bronchoalveolar Lavage. To obtain BALF, animals under sodium barbiturate anesthesia were killed by exsanguination. Lungs were lavaged *in situ* three times with a total of 5 mL 0.15 M saline heated to 37°C. The volume of fluid recovered was between 80 and 90% (85% on average) of the fluid introduced. An aliquot was removed

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and analyzed for total cell counts in a Bürker's chamber. The cells were then removed by centrifugation at 300*g* for 10 min, and the cell pellet was resuspended in 1 mL of saline. A cytospin preparation of the cells was stained by the method of Wright-Giemsa and a differential cell count made. Cytologic parameters were determined on post-treatment days 1 and 15.

Investigations. Animals were sacrificed on post-treatment days 1, 5, and 15 to measure the following parameters: *a*) lung weight coefficient (organ weight in mg/100 g body weight); *b*) BALF: total cell number $\times 10^5$/mL, differential cell count, including alveolar macrophages (AM), lymphocytes, and neutrophils (PMN) as a percentage, activity of the enzymes lactate dehydrogenase (LDH), alkaline phosphatase (AlP), and acid phosphatase (AP) by the method of Bergmeyer (*15*), and protein amount by the method of Lowry (*16*). Enzyme activities are expressed as units/mL. One unit is defined as 1 nmole substrate converted per minute. Total protein content is expressed as mg/mL of BALF.

Statistics. Experimental data were statistically treated by the Student's *t*-test ($p < 0.05$). Concurrently, the type of the combined effect was quantitatively determined by the method of Rothman (*14*). Results are presented in the tables as mean ± SE. Figures show changes as percentage versus control levels.

Reagents. The reagents used in biochemical and cytologic investigations were purchased from Fluka Chemie AG, Switzerland.

Results

Animal mortality to day 15 after sole exposures amounted to 8% with 4 Gy radiation and 21% with cadmium acetate. In the case of combined treatment with both agents (group 4), mortality attained 60.7% (synergistic effect).

The experimental data on lung weight coefficient and BALF cytology are summarized in Table 1. The lung-weight coefficient was increased at the three time points studied for cadmium alone as well as for cadmium combined with radiation. The increase by combined treatment (group 4) was of synergistic nature.

Percentages of AM, lymphocytes, and PMN were not significantly different from controls after exposure to radiation alone. Cadmium acetate, either alone or combined with radiation, reduced the percentage of AM and of lymphocytes and caused a sharp increase in the percentage of PMN on day 1 after treatment. By day 15, these changes were found to be less marked.

The experimental data for BALF biochemistry are shown in Table 2. LDH activity in BALF remained unaffected by radiation alone. Cadmium acetate alone or combined with radiation produced an elevation of LDH activity at the three time points studied. The group exposed to both agents combined showed a synergistic interaction (Fig. 1).

AlP activity in BALF was elevated on day 15 after sole radiation exposure and on days 1, 5, and 15 after sole cadmium acetate intake. Both agents combined produced synergistic elevations of AlP activity on days 1 and 5 (Fig. 2). AP activity in BALF was elevated on day 15 after 4 Gy irradiation and on days 1 and 15 after cadmium acetate given alone. Both agents combined caused elevations of enzyme activity on days 1, 5, and 15. At the first two time points, the increases were indicative of synergistic interaction (Fig. 3).

BALF protein contents were increased on days 1 and 5 for animals receiving cadmium acetate alone as well as those treated with both agents combined. Changes in the combination treatment group (group 4) on days 1 and 5 were synergistic in nature (Fig. 4).

Table 1. Effect of separate and combined treatments with cadmium and ionizing radiation on the weight coefficient of lungs and cytological indices.

Day after treatment		Weight coefficient of the lungs[a]	Total cell no. $\times 10^5$ mL BALF	AM, %	Lymphocytes, %	PMN, %
1	Control	673 ± 12.6[b]	10.2 ± 1.8[b]	91.2	5.2	3.6
	4 Gy	787 ± 81.2	10.9 ± 2.8	92.5	4.0	3.5
	Cd	1223 ± 114.2	11.3 ± 3.3	11.5	2.0	86.5
	Cd + 4 Gy	1547 ± 166.4*	5.4 ± 0.7*	12.0	2.5	85.5
5	Control	676 ± 18.9	–	–	–	–
	4 Gy	701 ± 36.2	–	–	–	–
	Cd	1250 ± 151.8*	–	–	–	–
	Cd + 4 Gy	1408 ± 117.8*	–	–	–	–
15	Control	606 ± 17.0	10.2 ± 1.0	88	8	4.0
	4 Gy	608 ± 39.2	11.3 ± 1.8	90	6.5	3.5
	Cd	1103 ± 88.9*	11.7 ± 2.6	45.5	11.5	43
	Cd + 4 Gy	1155 ± 126*	10.6 ± 1.0	54	1.0	45

Abbreviations: BALF, bronchoalveolar lavage fluid; AM, alveolar macrophages; PMN, neutrophils.
[a]Values are mg/100 g body weight.
[b]Values represent means ± SE of six animals.
*Different from control at $p < 0.05$, Student's *t*-test.

Table 2. Effect of separate and combined treatments with Cd and ionizing radiation on the enzyme activities and total protein content in BALF.[a]

	Day after treatment											
	1				5				15			
Parameter	Control	4 Gy	Cd	Cd+4 Gy	Control	4 Gy	Cd	Cd+4 Gy	Control	4 Gy	Cd	Cd+4 Gy
LDH, unit/mL	68±1.34	70±5.4	176±30.6	225±13.1*b	82±1.21	82±1.15	175±17.5*	200±28.8*b	66±12.0	84±7.5	97±7.6*	146±18.0*b
AlP, unit/mL	2.94±0.42	2.46±0.45	11.31±1.24*	13.71±1.28*b	2.59±0.38	2.61±0.32	4.59±0.55*	5.17±0.60*b	3.41±0.16	4.42±0.16	5.31±0.39*	5.73±0.57*
AP, units/mL	2.32±0.3	2.64±0.04	5.36±0.64*	6.4±0.52*b	2.48±0.33	2.34±0.24	1.96±0.19	5.40±0.53*b	2.06±0.15	2.58±0.18*	2.62±0.38*	3.34±0.34*
Total protein, mg/mL	0.42±0.04	0.39±0.03	2.13±0.24	2.76±0.31*b	0.45±0.04	0.41±0.04	0.67±0.06*	1.12±0.08*b	0.36±0.05	0.39±0.02	0.45±0.05	0.44±0.07

Abbreviations: LDH, lactate dehydrogenase, AlP, alkaline phosphatase; AP, acid phosphatase.
[a]The values represent mean ± SE of six animals.
[b]Synergic effect by Rothman's method (*14*).
*Different from control at $p<0.05$ by Student's *t*-test.

Discussion

The study was designed to identify the type of combined biological effects produced in the lungs by concurrent exposure to cadmium acetate and ionizing radiation. Both agents were used at a high dosage and synergetically increased animal death rate over the observation period, that is, within 15 days after treatment.

Our findings indicate that intratracheal cadmium acetate administered at 0.5 mg/kg body weight results in sharp increases in BALF activities of LDH, AlP and AP as well as in BALF protein contents. Over the period observed, the enzyme activities studied displayed a similar pattern of change with time, notably, a large rise over controls on day 1, and a distinct trend to decline on days 5 and 15.

The lavage fluid parameters we examined are generally accepted biochemical markers for toxic lung lesions that have been extensively used in experimental toxicology in the past 10–15 years. As demonstrated by a number of investigators, these parameters enable the demonstration of a cytotoxic effect, from increased membrane permeability to frank cell lysis (LDH), impaired or increased secretion by type-2 pneumocytes (AlP), enhanced phagocytic activity or cell killing (AP), and damage to the alveolar capillary barrier [protein (*17–23*)]. In the light of this information, the cadmium acetate dose used in our experiment apparently caused toxic damage to lung cells secreting the enzymes studied into broncoalveolar spaces and increased the permeability of capillary-alveolar membranes. Increases in PMN counts and in the lung-weight coefficient were evidence of an acute inflammatory process developing promptly on administration of cadmium acetate. This was the first phase of toxic lung response to cadmium, which proceeds with massive edema, disruption of alveolar surfaces, and PMN infiltration (*8–10,12*).

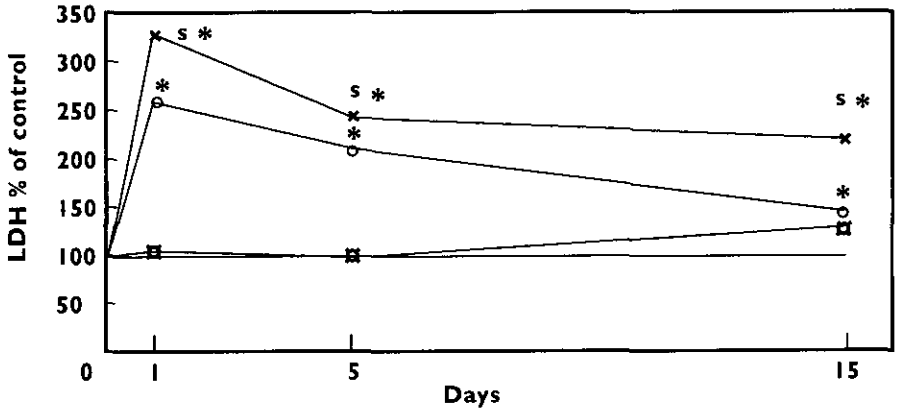

FIGURE 1. Time-dependent effect of separate and combined treatments with cadmium and ionizing radiation on lactate dehydrogenase (LDH) activity in bronchoalveolar lavage fluid. Each point is the mean ± SE for six rats, expressed as percentage of respective control value. The mean of control group LDH was between 66 and 82 units/mL during the 15-day study period. ( ) 4 Gy, (O) CD, (×) Cd + 4 Gy. (*) Significantly different from control at $p<0.05$ by Student's *t*-test, (s) synergic effect by Rothman's method (*14*).

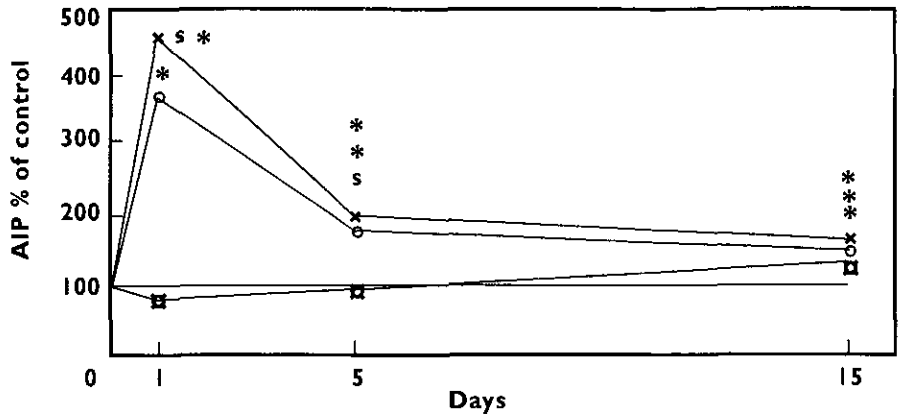

FIGURE 2. Time-dependent effect of separate and combined treatments with cadmium and ionizing radiation on alkaline phosphatase (AlP) activity in bronchoalveolar lavage fluid. Each point is the mean ± SE for six rats, expressed as percentage of respective control value. The mean of control group AlP was between 2.59 and 3.41 units/mL during the 15-day study period. ( ) 4 Gy, (O) CD, (×) Cd + 4 Gy. (*) Significantly different from control at $p<0.05$ by Student's t-test, (s) synergic effect by Rothman's method (*14*).

With 4 Gy γ irradiation alone, no substantial changes were noted in the parameters investigated. It was not until day 15 that AlP and AP activities were seen to rise. This is the time when radiation-damaged tissues regenerate, and elevation in the levels of the two enzyme markers probably reflects intensification of metabolic processes in the lungs (*24,25*).

Treatment with cadmium acetate and radiation combined resulted in changes that were more pronounced than in the case of either agent given alone. In group 4 (combination treatment), LDH activity rose over the control level by 331% on day 1, 243% on day 5, and 221% on day 15. The elevation significantly exceeded that seen with isolated treatments and, estimated by the method of Rothman (*14*), was indicative of synergistic interaction. On days 1 and 5, synergistic increases were also observed for the remainder of biochemical markers (AlP, AP, and protein). Additional support for severe lung damage came from more marked increases in the lung-weight coefficient on days 1 and 5 and reduction of BALF total cell counts on day 1.

Our experimental findings are in agreement with studies by others on combined lung lesions from exposure to chemical agents and radiation. In 1980, Haschek et al. (*26*) showed that external irradiation with 2 or 4 Gy X-rays potentiated lung collagen deposition due to butylated hydroxytoluene. Enhancement of pneumotoxicity has been reported for simultaneous treatment with some cytostatics (cyclophosphamide, bleomycin, etc.) and ionizing radiation (*4–7*).

This work was not aimed at disclosing mechanisms underlying synergistic lung damage, and these mechanisms remain unclear. Possibly, combined treatment increases the severity of damage to the organ by intensifying lipid peroxidation in lung parenchyma. This is suggested by the fact that both agents are known to generate active oxygen species, an effect that would be expected to be enhanced in the case of combined treatment (*27–29*).

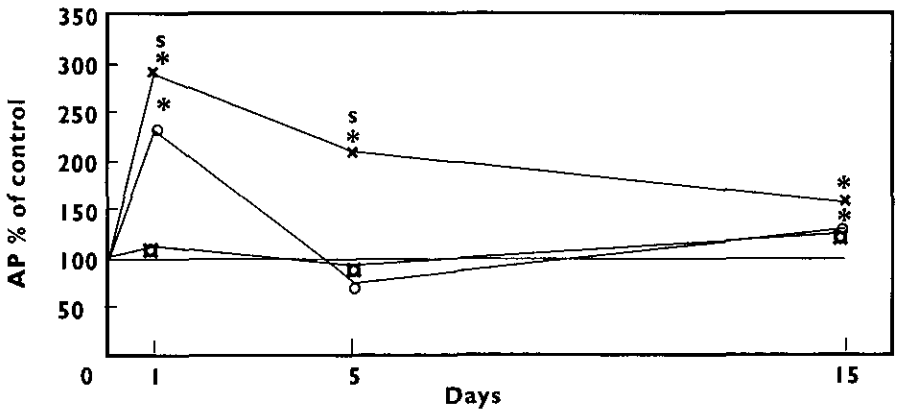

FIGURE 3. Time-dependent effect of separate and combined treatments with cadmium and ionizing radiation on acid phosphatase (AP) activity in bronchoalveolar lavage fluid. Each point is the mean ± SE for six rats, expressed as percentage of respective control value. The mean of control group AP was between 2.06 and 2.42 units/mL during the 15-day study period. ( ) 4 Gy, (O) CD, (×) Cd + 4 Gy. (*) Significantly different from control at $p<0.05$ by Student's t-test, (s) synergic effect by Rothman's method (*14*).

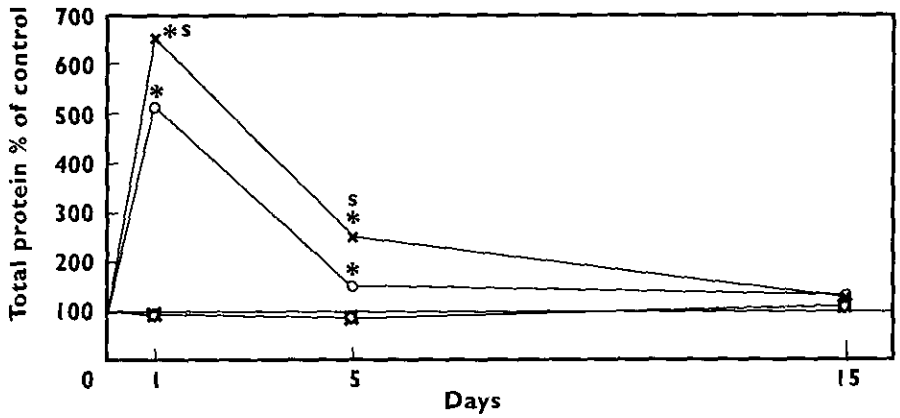

FIGURE 4. Time-dependent effect of separate and combined treatments with cadmium and ionizing radiation on total protein content in bronchoalveolar lavage fluid. Each point is the mean ± SE for six rats, expressed as percentage of respective control value. The mean of control group total protein content was between 0.36 and 0.46 mg/mL during the 15-day study period. ( ) 4 Gy, (O) CD, (×) Cd + 4 Gy. (*) Significantly different from control at $p<0.05$ by Student's t-test, (s) synergic effect by Rothman's method (14).

In conclusion, it may be stated that external whole-body 4 Gy irradiation was observed to potentiate a number of toxic cadmium acetate effects in the lungs. The BALF biochemical markers, LDH, AlP, AP, and protein, appear to be reliable indicators for identifying the type of lung response to chemical agents and radiation combined and can serve as valuable tools in exploring this area of environmental medicine.

REFERENCES

1. Streffer, I. C., and Muller, W.-U. Radiation risk from combined exposure to ionizing radiation and chemicals. In: Advances in Radiation Biology, Vol. 11. Academic Press, Essen, 1984, pp. 173–205.
2. Müller, W.-U., and Streffer, C. Risk to preimplantation mouse embryos of combinations of heavy metals and radiation. Int. J. Radiat. Biol. 51: 997–1006 (1987).
3. Tiunov, L. A., Gerbin, E. A., and Gerdin, B. N. Radiation and Poisons. Atomizdat, Moscow, 1977.
4. Einhorn, L., Krause, M., Hornback, N., and Furnas, B. Enhanced pulmonary toxicity with bleomycin and radiotherapy in oat-cell lung cancer. Cancer 37: 2414–2416 (1977).
5. Steel, G. G., and Peckam, J. Lung damage in $C_{57}Bl$ mice following thoracic irradiation: enhancement by chemotherapy. Br. J. Radiol. 52: 741–747 (1979).
6. Collis, C. H., Down, J. D., Pearson, A. E., and Steel, G. G. Bleomycin and radiation-induced lung damage in mice. Br. J. Radiol. 56: 21–26 (1983).
7. Varekamp, A. E., de Vries, A. J., and Hagenbeek, A. Lung damage in the rat after irradiation and treatment with cytotoxic drugs. Br. J. Cancer 53: 347–350 (1986).
8. Palmer, K. C., Snider, G. L., and Hayes, J. A. An association between alveolar cell proliferation and interstitial fibrosis following acute lung injury. Chest 69: 307–309 (1976).
9. Straus, R. H., Palmer, K. C., and Hayes, J. A. Acute lung injury induced by cadmium aerosol. I. Evolution of alveolar cell damage. Am. J. Pathol. 84: 561–578 (1976).
10. Lee, J. S., and White, K. L. A review of the health effects of cadmium. Am. J. Ind. Med. 1: 307–317 (1980).
11. Dudley, R. E., Svoboda, D. J., and Klaassen, C. D. Acute exposure to cadmium causes severe liver injury in rats. Toxicol. Appl. Pharmacol. 65: 302–313 (1982).
12. Kobrle, V., Mirejovska, E., Holusa, R., and Hurych, J. Changes in pulmonary connective tissue proteins after a single intratracheal instillation of cadmium chloride in the rat. Environ. Res. 40: 3–14 (1986).
13. Snider, G. L., Hayes, J. A., Korthy, A. L., and Lewis, G. P. Centrolobular emphysema experimentally induced by cadmium chloride aerosol. Am. Rev. Respir. Dis. 108: 40–48 (1973).
14. Rothman, K. J. Synergy and antagonism in case effect relationships Am. J. Epidemiol. 99: 385–388 (1974).
15. Bergmeyer, H. U., Gawehn, K., and Grassl, M. Enzymes as biochemical reagents. In: Methods of Enzymatic Analysis (H. U. Bergmeyer, Ed.), Academic Press, New York, 1974, pp. 425–522.
16. Lowry, O. H., Resenbrough, N. J., Farr, N. J., and Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275 (1951).
17. Tierney, D. F., Yang, J., Ayers, L. Enzymatic activity in rat lungs. Chest 67: 408–428 (1975).
18. Henderson, R. F., Rebar, A. H., and de Nicola, D. B. Early damage indicators. IV. Biochemical and cytologic response of the lung to lavage with metal salt. Toxicol. Appl. Pharmacol. 51: 129–135 (1979).
19. Hook, G. E. R. Extracellular hydrolases of the lung. Biochemistry 17: 520–528 (1978).
20. Henderson, R. F. Use of bronchoalveolar lavage to detect lung damage. Environ. Health Perspect. 56: 115–129 (1984).
21. Smith, L. L., Cohen, G. M., and Aldridge, W. N. Morphological and biochemical correlated of chemical induced injury in the lung. Arch. Toxicol. 58: 214–218 (1986).
22. Hu, P. H., Miller, F. J., Daniels, M. J., Hatch, G. E., Graham, Gardner, D. E., and Selgrade, M. K. Protein accumulation in lung lavage fluid following ozone exposure. Environ. Res. 29: 377–388 (1982).
23. Muller, B., and von Wichert, P. Bronchoalveolar lavage protein. Klin. Wochenschr. 63: 781–787 (1985).
24. Gross, N. J. The pathogenesis of radiation-induced damage. Lung 159: 115–125 (1981).
25. Penney, D. P., Silmann, D. W., Rubin, Shapiro, D. L., Finkelstein, J., and Cooper, R. A. Morphologic changes reflecting early and late effects of irradiation of the distal lung of the mouse: a review. Scan. Electron Microsc. 1: 413–425 (1982).
26. Haschek, W. M., Meyer, K. R., Ullrich, R. L., and Witchi, H. P. Potentiation of chemically induced lung fibrosis by thorax irradiation. Int. J. Radiat. Oncol. Biol. Phys. 6: 449–455 (1980).
27. Hussain, T., Shukla, G. S., and Chandra, S. V. Effects of Cd on superoxide dismutase and lipid peroxidation in liver and kidney of grow in rate: in vivo and in vitro studies. Pharmacol. Toxicol. 60: 355–359 (1987).

28. Frank, L. Superoxide dismutase and lung toxicity. Trends Pharmacol. Sci. 3: 124–128 (1983).
29. Shukla, G. S., Hussain, T., Srivastava, R. S., and Chandra, S. V. Glutathion peroxidase and catalase in liver, kidney, testis, and brain regions of rats following cadmium exposure and subsequent withdrawal. Ind. Health 27: 59–69 (1989).